A METHOD OF DETERMINING THE SURFACE AREA OF COTTONSEED

Production Research Report No. 105

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

PREFACE

During the past several years, cotton breeders, geneticists, physiologists, and other cotton research workers have demonstrated increasing interest in cottonseed sizes and dimensions and have been applying such information in some of their research investigations.

As described in this report, the method for determining the surface area of cottonseed was originally developed in 1932–33 and applied in research studies at the U.S. Cotton Ginning Research Laboratory, Stoneville, Miss. The method was recorded in the form of an office report, but was never published. Recent investigation has failed to reveal the subsequent development of a similar laboratory method.

Publication of this method in bulletin form makes the method widely available to research workers.

The main purpose of this report is, therefore, not to present measurement data on particular cottonseed varieties used in the study, since many of the varieties used are now obsolete, but rather to describe and demonstrate a method of making cottonseed surface area determinations.

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Washington, D.C. Issued March 1969

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington, D.C. 20402—Price 10 cents

A METHOD OF DETERMINING THE SURFACE AREA OF COTTONSEED

By CHARLES S. SHAW, *research cotton technologist, Agricultural Engineering Research Division, Agricultural Research Service* [1]

Studies relating cottonseed size to ginning performance and the quantity of linters left on cottonseed at ginning, prompted researchers at the U.S. Cotton Ginning Research Laboratory, Stoneville, Miss., to devise a practicable method of determining the surface area of cottonseed.

PREVIOUS METHODS

The following three methods were investigated by the author and were found to be either impractical or inaccurate.

Geometrical measurement. Direct geometrical measurements of cottonseed and calculation of the area from these measurements are made by treating each seed as a sphere, a cylinder, and a cone; finding the surface area of each body; and then averaging the three results. These measurements, however, give only a rough approximation of the true surface area.

Velocity in liquid. In this method, a delinted seed is dropped into a liquid of known, uniform density, the principle being that the area of an object (in this case, a delinted seed) is directly proportional to its velocity as it falls through the liquid. This relationship, however, holds true only for regular shaped objects of uniform density. Obviously, a cottonseed does not meet these requirements.

Oil immersion. The procedure in the oil immersion method[2] is to immerse the sample of cottonseed in paraffin oil, and then centrifuge it until the excess oil is thrown off. A thin film of oil adheres to the seed coat and, as the density of the oil is known, the surface area of the seed is calculated from the increase in weight of the sample. This method is impractical because even the best high-speed centrifuging machine may not leave a uniform film of oil on the seed. Furthermore, the oil is difficult to remove from the interstices between the seeds and from the depressions in the seed coat.

Other cottonseed measurement studies have been reviewed. However, because a description of the

[1] George W. Pfeiffenberger, deceased, formerly cotton technologist, U.S. Department of Agriculture, collaborated with the author in the earlier research.

[2] This method was described in an article by R. L. Narasimha Ayyanger, in the Madras Agricultural Department Year Book of 1929.

method given in the report had not been published, the method was either not known or not used in these other studies.[3]

DEVELOPMENT OF THE STONEVILLE METHOD

The Stoneville method, as it is described in this report, is based on the fact that the outer coat of a cottonseed is of uniform thickness, and, therefore, the total surface area may be calculated from the average weight per unit area of flat sections of the seed coat. A series of preliminary experiments was run to demonstrate the uniform thickness of the seed coat, and the following procedure was used.

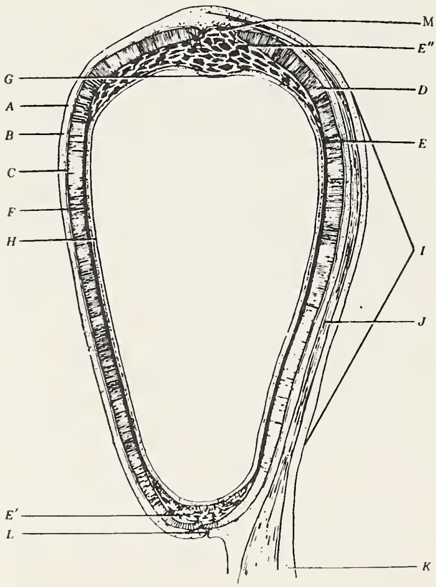

FIGURE 1.—Longitudinal section through the mature cottonseed: *A*, outer epidermis; *B*, outer pigment layer; *C*, colorless layer; *D*, palisade layer; *E* and *E′*, inner pigment layer, differentiated at microphylar and *E″*, chalazal cap; *F*, fringe cells, *I*, raphe; *J*, vascular bundle; *K*, funiculus; *L*, microphyle; *M*, chalazal aperture. (Adapted from Leahy.)

First the cottonseed was delinted with sulfuric acid. Then the seed was cut in two about halfway between the proximal and distal ends, and the kernel or seed meat was easily removed. Inside the empty hull of each cottonseed is a thin membrane. Attached to this membrane at the larger or distal end of the seed, a small knot, known as the chalazal cap (fig. 1),[4 5] was easily removed with a pair of tweezers. To determine whether or not the seed coat was of uniform thickness, the author cut the empty coat into several small, flat pieces, selected five pieces, and measured each piece with a micrometer caliper.

The results of these preliminary measurements on one variety of cotton are shown on table 1. The letters, A, B, C, D, E, represent the small pieces chosen from different sections of the seed. Note

[3] Porterfield, Jay, and Smith, Edward M. PHYSICAL CHARACTERISTICS AND FIELD PERFORMANCE OF MECHANICALLY GRADED ACID DELINTED COTTONSEED. Okla. A & M Col. Expt. Sta. Tech. Bul. T-60, 24 pp. 1956.

Hodson, E. A. LINT FREQUENCY IN COTTON WITH A METHOD FOR DETERMINATION. Ark. Agr. Expt. Sta. Bul. 168, 11 pp. 1920.

Thurman, R. L., and Henderson, M. T. THE INHERITANCE OF LINT DENSITY INDEX IN AMERICAN UPLAND COTTON. Agron. Jour. 48: 344-347. 1956.

[4] Christiansen, Meryl N., and Moore, R. P. SEED COAT STRUCTURE DIFFERENCES THAT INFLUENCE WATER UPTAKE AND SEED QUALITY IN HARD SEED COTTON. Agron. Jour. 51: 582-584. 1959.

[5] Appreciation is expressed by the author to Meryl Christiansen and R. P. Moore for their courtesy in permitting the use of the diagram shown in fig. 1.

that the maximum difference in thickness was not over 0.025 millimeters, which is negligible for this work. Identical series of tests were run on 16 varieties of cottonseed, and for each variety the thickness of the coat was found to be uniform for all sections of each seed. The thickness varied, of course, from seed to seed in the same variety, but there were no distorting measurable differences in one seed. With this fact established, a reasonably sound basis for subsequent experiments was obtained.

TABLE 1.—*Example of thickness measurements made on seed coats from five different sections of each seed on samples taken from Missdel W. R. cotton, first variety, crop of 1932*

Seed No.	Thickness of seed coat sections					Average thickness per sample
	A	B	C	D	E	
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	0.229	0.216	0.216	0.205	0.229	0.219
2	.229	.229	.206	.216	.229	.221
3	.203	.190	.203	.203	.203	.200
4	.216	.203	.198	.203	.203	.201
5	.229	.229	.241	.229	.229	.231

MECHANICS OF THE STONEVILLE METHOD

The following steps outline the mechanics of the Stoneville method of obtaining the surface area of cottonseed:

1. First delint the seed sample by pouring about 10 cc. of commercial sulfuric acid over a 50-g. sample and stirring vigorously. When the linters have disappeared, strain off the excess acid, wash the seed thoroughly, and dry them. Then mix the seed, quarter them, and quarter them again to provide a representative sample. At random, select any number of seeds for the tests. (In the preliminary work, the author considered 10 seeds as being sufficiently representative of the sample after successive quartering had reduced their number.)

2. Cut each seed in two with a sharp razor blade or scalpel. The edge of blade must be keen or it may crack the brittle seed coat. Usually, the kernel drops out by itself when the two parts of the coat are separated, but if not, you may easily remove the kernel with a small pair of tweezers.

3. Remove the membrane and knot (chalazal cap) from the hull.

4. Accurately weigh the empty hull and record the weight as shown in the third column of table 2. Tests identical to the one recorded on table 2 were made on several other cotton varieties, with similar results.

5. Examine the outside of the empty hull for small, flat areas, and cut them out from the hull.

Choose only the larger, flat pieces for cutting because they are much easier to measure and weigh.

6. Trim each flat piece to a rectangular shape. Then, obtain the area of the rectangle by measuring its length and breadth with a micrometer caliper. You can obtain the surface area, as represented by one unit of weight, by first weighing the piece accurately and then dividing the area of the piece by its weight. To simplify the calculations, express the figures in area per unit weight rather than in weight per unit area. The unit weight is 1 mg., and the area is expressed in square millimeters. As the seed coat has been proved sufficiently uniform in thickness throughout all parts of each seed, this area per unit weight is applicable to the whole surface area as well as to the small, flat piece accurately measured and weighed. Thus, the total weight of the empty hull multiplied by the area per unit weight gives the total surface area of the seed.

TABLE 2.—*Example of data obtained in applying the weight and measurement method of determining surface area of cottonseed*

Seed sample No.	Total weight of seed	Weight of empty hull	Area of flat piece	Weight of flat piece	Area per 1 mg.	Total surface area of seed
	Mg.	*Mg.*	*Sq. mm.*	*Mg.*	*Sq. mm.*	*Sq. mm.*
1	97.0	32.0	3.29	1.00	3.29	105.28
2	125.0	40.0	3.47	.90	3.86	154.40
3	116.5	38.2	4.03	1.00	4.03	153.95
4	109.0	35.8	2.06	.70	2.94	105.25
5	119.7	42.5	4.39	1.10	3.99	170.00
6	115.0	37.0	3.70	.80	4.62	170.94
7	108.0	36.8	3.35	1.00	3.35	123.28
8	95.0	35.0	3.87	1.00	3.87	135.45
9	112.0	36.0	2.92	.70	4.17	150.12
10	116.0	38.0	3.45	.90	3.83	145.54

USE OF DIE PUNCH FOR FLAT AREAS

For a large volume of work a steel die punch may be used to stamp out the flat pieces. Such a die was made and used at Stoneville (figs. 2 and 3) to stamp out seed coat disks 2 mm. in diameter. The die should have a sharp cutting edge and be so accurately made that the area of seed coat cut by it will be a definite size. This will eliminate the need for further trimming of the piece as well as the possibility of errors that result from using pieces whose areas are not exactly the same.

Although the shape of the die would depend chiefly on its ease of manufacture, a circular die would eliminate corners on the stamped out piece and thereby lessen the danger of breaking or chipping. The area of the piece should be about 3 mm. because pieces smaller than this would be difficult to handle and weigh; whereas, if the die were made much larger, you would be less likely to find flat pieces as large as the die.

By using a die punch, you eliminate one-half of the calculations (table 3). Since the area stamped out is constant, only the weight per unit area is needed. In addition, a die punch will provide more flat pieces per seed than can be obtained from hand cutting.

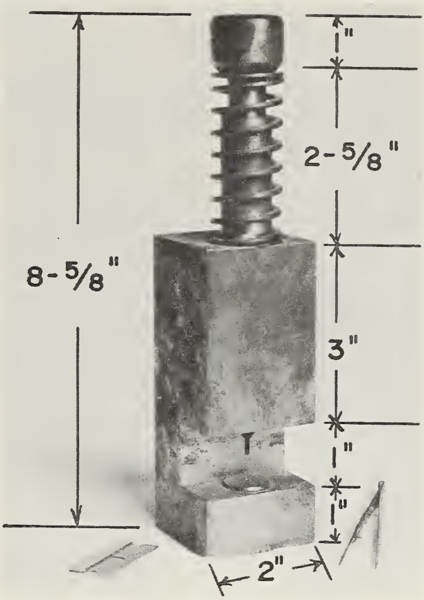

FIGURE 2.—Die punch for punching circular segments of the cottonseed coat.

FIGURE 3.—Cottonseed during stages of preparation for surface-area determination: *A*, After ginning; *B*, delinted; *C*, kernel removed; *D*, seed coat divided; *E*, flat pieces of coat stamped by die.

TABLE 3.—*Cottonseed surface-area determinations on 16 foundation varieties made with the aid of a die punch, on the basis of weight per unit area, crop of 1936* [1]

Cottonseed variety	Dry weight of 10 delinted seeds	Weight of 10 empty hulls	Weight of 50 small punched pieces	Area of 50 small pieces [2]	Area of one seed [3]
	Mg.	*Mg.*	*Mg.*	*Sq. mm.*	*Sq. mm.*
Cleveland	935	352	50.5	157.08	109.49
Triumph 44	954	324	48.5	157.08	104.94
Rowden	1,069	367	49.5	157.08	116.46
Half & Half	886	291	46.0	157.08	99.37
Stoneville	874	322	52.3	157.08	97.05
Mex. B. B.	1,060	356	48.5	157.08	115.30
Wilds	1,089	378	48.7	157.08	121.92
Dixie Triumph	916	324	50.2	157.08	101.38
Startex	993	347	48.8	157.08	111.69
Cook	791	287	49.7	157.08	90.71
Ark. 17	961	348	50.5	157.08	108.24
Farm Relief	1,026	393	51.3	157.08	120.34
Acala	980	349	49.8	157.08	110.08
Missdel	912	315	46.8	157.08	105.73
Qualla	1,039	387	50.8	157.08	119.66
D. & P. L.	893	304	47.7	157.08	100.11

[1] Average of three replications.
[2] The area of each small punched disk = 3.1416 mm^2.
[3] Formula for calculation of surface area:

$$\frac{\text{Weight of 10 empty hulls} \times \text{area of 50 small pieces}}{\text{Weight of 50 small punched pieces} \times 10} = \text{Surface area of one seed.}$$

SUMMARY

Studies at the U.S. Cotton Ginning Research Laboratory, Stoneville, Miss., relating cottonseed size to ginning performance and the quantity of linters left on cottonseed at ginning, prompted researchers to devise a practical laboratory method of determining the surface area of cottonseed. The method is based on the fact that since the outer coat of each cottonseed is of uniform thickness, the total surface area can be calculated from the weight per unit area of flat sections of the seed coat.

The Stoneville method for making cottonseed surface area determinations is as follows:

1. Delint the seed with acid.
2. Cut each seed to be measured in two, and remove the meat kernel, chalazal cap, and membrane.
3. Weigh the remaining empty hull.
4. Cut or punch out small, flat areas of the empty hull and weigh them. If a punch is used, areas of the flat, punched out disks are predetermined; however, if a punch is not used, cut out the flat pieces in a rectangular shape and measure them with a micrometer caliper.
5. To calculate the surface area of the cottonseed, divide the total weight of the empty seed hull by the per unit weight and then multiply this answer by the per unit area.

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